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# Elevated biomarkers of endothelial dysfunction/activation at ICU admission are associated with sepsis development

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ABSTRACT

Widespread endothelial activation and dysfunction often precede clinical sepsis. Several endothelium-related molecules have been investigated as potential biomarkers for early diagnosis and/or prognosis of sepsis, providing different results depending on study designs. Such factors include endothelial adhesion molecules like E- and P-selectin, and the intercellular adhesion molecule-1, vascular endothelial cadherin, growth factors such as Angiopoietin-1 and -2 and vascular endothelial growth factor, as well as von Willebrand factor antigen. We sought to investigate whether circulating biomarkers of endothelial activation/dysfunction measured at ICU admission are associated with subsequent sepsis development.

Eighty-nine critically-ill patients admitted to a general ICU who met no sepsis criteria were studied. Plasma or serum levels of the above-mentioned endothelium-derived molecules were measured during the first 24 h post ICU; acute physiology and chronic health evaluation (APACHE) II and sequential organ failure assessment (SOFA) scores, age, sex, diagnostic category, and circulating procalcitonin (PCT) and C-reactive protein (CRP) levels were additionally measured or recorded.

Forty-five patients subsequently became septic and 44 did not. Soluble (s) E- and P-selectin levels, circulating PCT, SOFA score and diagnostic category were significantly different between the two groups. Multiple logistic regression analysis associated elevated sE- and sP-selectin levels and SOFA with an increased risk of developing sepsis, while multiple Cox regression analysis identified sE- and sP-selectin levels as the only parameters related to sepsis appearance with time [RR = 1.026, 95%CI = 1.008–1.045, $p = 0.005$; RR = 1.005 (by 10 units), 95%CI = 1.000–1.010, $p = 0.034$, respectively]. When trauma patients were independently analyzed, multiple Cox regression analysis revealed sE-selectin to be the only molecule associated with sepsis development with time (RR = 1.041, 95%CI: 1.019–1.065; $p < 0.001$).

In conclusion, in our cohort of initially non-septic critically-ill patients, high levels of the circulating endothelial adhesion molecules E- and P-selectin, measured at ICU admission, appear to be associated with sepsis development in time.



## 1. Introduction

The vascular endothelium is composed of a single layer of squamous cells that cover the inner surface of blood vessels. While in the past it was believed to be nothing more than an inert divider, in the last decades it has been found that the endothelium is a separate, highly metabolically active organ that plays an important role in organogenesis, tissue homeostasis and immune response [1–3].

Excessive and sustained activation of the endothelium resulting in increased vascular permeability is a crucial process during sepsis [4,5]. The septic syndrome is characterized by a systemic inflammatory response in the effort of the organism to eliminate/control infections [6]. A common element of severe infections is the very

*Abbreviations:* Ang-1, angiopoietin-1; Ang-2, angiopoietin-2; APACHE, acute physiology and chronic health evaluation; aPTT, activated partial thromboplastin time; BMI, body mass index; BWP, biphasic waveform; CI, confidence interval; CRP, C-reactive protein; ELISA, enzyme-linked immunosorbent assay; $FiO_2$, fraction of inspired oxygen; ICAM-1, intercellular adhesion molecule 1; ICU, intensive care unit; IQR, interquartile range; OR, odds ratio; $PaO_2$, partial pressure arterial oxygen; PCT, procalcitonin; RR, relative risk; SIRS, systemic inflammatory response syndrome; SOFA, sequential organ failure assessment; VE-cadherin, vascular endothelial cadherin; VEGF, vascular endothelial growth factor; vWF, von Willebrand factor.

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acute, potent and generalized activation of the immune and hemostatic system [3,7]. This may result in extensive inflammation and vascular thrombosis, a microcirculatory disorder that has as a consequence the disruption of smooth blood flow and hence oxygen toward the cells [3,5,7].

Despite the significant progress of the last decades in the investigation of sepsis mechanisms, mortality due to severe sepsis remains high. Recent data pinpoint endothelial dysfunction as a very important pathogenetic factor [8]. Endothelial activation often precedes endothelial dysfunction, and a large number of endothelial cell (EC) active molecules have been investigated as potential biomarkers for the early diagnosis and prognostication of sepsis [9]. These biomarkers include regulators of endothelial activation, adhesion molecules, as well as mediators of permeability, vasomotor tone, and coagulation [4]. However, as yet there is no generally acceptable way of either diagnosing endothelial dysfunction at its early phase or associating the latter with the septic process [10].

The aim of this study was to determine the role of endothelial biomarkers in the timely diagnosis of sepsis. We evaluated whether elevated levels of circulating endothelial biomarkers of critically-ill patients upon admission to the Intensive Care Unit (ICU) can predict sepsis before it is clinically manifested, so the appropriate therapeutic handlings can be performed at an early phase in an effort to achieve optimal treatment.

## 2. Materials and methods

The study was approved by the Evangelismos Hospital Research Ethics Committee and all procedures carried out on patients were in compliance with the Helsinki Declaration. Informed written consent was obtained from all patients' next-of-kin prior to any study procedure.

### 2.1. Chemicals and reagents

Human soluble (s)P-selectin, sE-selectin, angiopoietin-1 (Ang-1), angiopoietin-2 (Ang-2), vascular endothelial cadherin (sVE-cadherin), vascular endothelial growth factor (VEGF) and intercellular adhesion molecule-1 (sICAM-1) were measured by ELISA assays purchased from R&D Systems (R&D Systems Inc., Minneapolis, MN, USA). The Imubind von Willebrand factor (vWF) ELISA was purchased from American Diagnostica (Sekisui Diagnostics, Sekisui Chemical Co. Ltd., Tokyo, Japan). All reagents used were of analytical grade.

### 2.2. Study population

Prior to enrolment, we screened all consecutive admissions to the Evangelismos Hospital ICU, Athens, Greece over a twenty-four month period for eligibility. Exclusion criteria were as follows: sepsis on or within the first 24 h of ICU admission according to the established sepsis criteria [6], BMI >35 kg/m$^2$, age <18 years, pregnancy, brain death, end-stage cancer, total ICU stay <3 days, readmission or transfer from another ICU, contagious diseases (human immunodeficiency virus, hepatitis) and oral intake of corticosteroids at an equivalent dosage of $\geqslant$1 mg/kg prednisone/day for a period of more than one month. Out of 177 non-septic subjects screened for eligibility over the study period, finally 89 (62 male and 27 female) critically-ill patients were enrolled in the study based on the above-mentioned criteria and consent to participate. The patients recruited in the study suffered from medical, surgical and trauma-related pathologies. Patients were considered to have sepsis when they developed systemic inflammatory response syndrome (SIRS) as a result of documented infection, septic patients with evidence of organ dysfunction were considered to have severe sepsis, and septic patients with persisting hypotension (despite adequate fluid resuscitation) were considered to have septic shock, in accordance with international guidelines and recommendations [6]. Clinical data and blood samples were obtained from the patients enrolled.

Following study enrolment, baseline (upon ICU admission) anthropometric data (age, sex, height, weight), procalcitonin (PCT) and C-reactive protein (CRP) plasma concentrations and detailed organ system-oriented medical history were recorded. A venous blood sample was drawn and processed as described in "Blood collection". All patients were followed daily for the presence and source of infection; other parameters recorded included vital signs, arterial blood gases, complete blood cell count and differential, serum chemistries, administered medications, mechanical ventilation features, and renal replacement. Patients were followed until discharge from the hospital or death. Patients were assigned to groups based on the presence (sepsis-positive, $N = 45$) or absence (sepsis-negative, $N = 44$) of sepsis at any point during their ICU stay. The patients who met the criteria for sepsis during the first 24 h post ICU admission were rejected from the study, to rule out subjects that might have had subclinical sepsis upon ICU entry.

### 2.3. Blood collection

Three milliliters (3 ml) of venous blood were collected within the first 24 h post ICU admission. Blood samples were either collected in red topped Vacutainer tubes (BD, Mississauga, Ontario, Canada) in order to collect serum, or in Vacutainer tubes containing either 0.129 M (3.8%) trisodium citrate or EDTA for the collection of plasma. Whole blood was allowed to clot by leaving it undisturbed at room temperature for 15–30 min. Serum was collected after centrifugation at 1000*g* for 10 min at 4 °C, apportioned into 0.5 ml aliquots, and stored at −80 °C until used. Alternatively, the citrate or EDTA-containing tubes were centrifuged for 10 min at 1000*g* at 4 °C to remove cells from plasma. Subsequently, plasma was collected, apportioned into 0.5 ml aliquots and stored at −80 °C until used.

### 2.4. Measurement of endothelial biomarkers

All factors were measured in either serum or plasma samples by enzyme-linked immunosorbent assay (ELISA), according to the manufacturers' instructions. The assays use two different polyclonal antibodies against the molecules as catching and tagging antibody.

### 2.5. CRP and PCT measurement

CRP was measured in plasma using an immunoturbometric assay (Tina-quart C-reactive protein, Roche Diagnostics GmbH, Mannheim, Germany). Plasma PCT levels were determined by means of a specific and ultrasensitive immunoluminometric assay (Liaison Brahms procalcitonin, Diagnostica, Berlin, Germany).

### 2.6. Statistical analysis

Data are presented as mean ± standard deviation (SD) for normally distributed variables or as median with inter-quartile range (Q1–Q3) for not normally distributed data. The two-group comparisons were made by the *t*-test or the non-parametric Mann–Whitney test for skewed data. Associations between qualitative variables were examined by the chi-square test or the Fisher's exact when appropriate. Kruskal–Wallis ANOVA followed by Dunn's multiple comparison post hoc test were used to examine differences between more than two groups. Multiple logistic regression analysis was performed to identify potential risk factors for sepsis development. The examined variables, which entered in